Osteo arthritis, aetiology, pathology and animal models

POS0371 BIOLOGICAL REPRODUCIBILITY OF TARGETED LIPIDOME ANALYSES IN PLASMA AND ERYTHROCYTES OVER A 6-WEEK PERIOD

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Background: Lipidomics analysis has become a valuable technology for understanding patho-physiological mechanisms and the identification of candidate biomarkers in rheumatic musculoskeletal disorders. Variability in within-subject repeated measurements may lead to bias towards the null when estimating the association between biomarkers and a disease or treatment. Hence, information regarding the stability of the metabolite levels over time is essential.

Objectives: We aimed to assess the lipid composition and biological reproducibility of lipid measurements in plasma and erythrocytes.

Methods: Plasma and erythrocyte samples from 42 osteoarthritis patients (77% women, mean age 65 years, mean BMI 27 kg/m²), obtained non-fasted at baseline and six weeks, were used for the quantitative measurement of up to 1000 lipid species across 13 lipid classes with the LipidizerTM platform in nmol/mL. Data was processed based on the relative standard deviation of quality controls, taking batch effects into account. Intraclass correlation coefficients (ICCs) and corresponding 95% confidence intervals (CI) were calculated to investigate the variability of the lipid concentrations between timepoints. The ICC distribution of lipid metabolites in plasma and erythrocytes were compared using two-sided paired Wilcoxon tests.

Results: We measured 778 lipids in plasma, compared to 916 lipids in erythrocytes. After data processing, the analyses included 630 lipids in plasma, and 286 in erythrocytes. From these, 243 lipids overlapped between sample types. Major differences were observed between the sample types in the number of lipids per lipid class and the total concentration of the lipids within a class. Triacylglycerols (TAG) and cholesteryl esters (CE) were more abundant in plasma. Conversely, phosphatidylethanolamines (PE), sphingomyelins (SM) and ceramides (CER) were less abundant in plasma compared to erythrocytes (table 1). In plasma 78% of lipid measurements were good to excellently reproduced, with an overall median ICC 0.69. Compared to plasma, a considerably lower amount (35%) of lipids were well reproduced in erythrocytes. Median reproducibility of lipids in erythrocytes was 0.51. Figure 1 shows the ICC score distribution in plasma with erythrocytes, with a significantly better reproducibility in plasma (p-value<0.001). However, while overall reproducibility was better in plasma, this was not observed for all lipid classes. At class-level, reproducibility in plasma was superior for TAGs and CEs, while CERs, DAGs, (L)PEs and SMs showed better reproducibility in erythrocytes.

Conclusion: In plasma biological reproducibility was good for most lipid measurements. Although overall reproducibility was better in plasma compared to erythrocytes, notable differences were observed at individual- and lipid class-level that may favour the use of a particular sample type.

Table 1. Number of individual lipids per class and class concentrations in plasma and erythrocytes

	Plasma		Erythrocytes	
	Number of lipid species	Class concentration (nmol/mL)	Number of lipid species	Class concentration (nmol/mL)
Triacylglycerols	482	1579.4 (1064.9-3195.2)	134	6.5 (5.6-9.4)
Diacylglycerols	9	13.3 (8.4-22.2)	10	5.8 (4.7-6.2)
Free fatty acids	20	745.3 (552.0-1202.9)	20	486.9 (379.2-669.2)
Cholesteryl esters	24	4571.6 (4065.1-5521.3)	5	1.2 (0.9-1.7)
Phosphatidylcholines	31	4013.7 (3203.1-4661.6)	42	3899.2 (3723.0-4296.6)
Phosphatidylethanolamines	26	156.2 (120.9-180.3)	42	3954.6 (3721.9-4323.3)
Lysophosphatidylcholines	9	385.9 (335.6-442.9)	7	119.8 (109.7-168.9)
Lysophosphatidylethanolamines	2	4.2 (3.5-4.9)	4	8.6 (6.8-9.7)
Sphingomyelins	12	1204.6 (1037.0-1351.9)	8	2695.8 (2434.8-2815.6)
Ceramides	6	14.1 (11.9-17.4)	7	163.0 (133.3-186.4)
Dihydroceramides	2	1.0 (0.8-1.3)	1	1.8 (1.4-2.1)
Hexosylceramides	5	5.1 (4.7-5.9)	4	5.6 (5.0-7.4)
Lactosylceramides	2	3.4 (2.7-3.8)	2	23.8 (20.6-33.5)

Numbers represent median (interquartile range) unless otherwise specified. Data represents baseline measurements.

Figure 1. The ICC score distribution of lipids measured in plasma and erythrocytes. Lipids are ranked ascending by plasma samples. A density plot of the ICC score distributions from plasma and erythrocyte samples is inserted in the lower right corner; the p-value represents the result from the Wilcoxon signed-rank test used to compare the distributions. The dashed lines correspond to the median ICC scores of lipids in plasma (blue) and erythrocytes (red).

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POS0372 LORECEVIVINT (SM04690), AN INTRA-ARTICULAR, SMALL-MOLECULE CLK2/DYRK1A INHIBITOR THAT MODULATES THE WNT PATHWAY, AS A POTENTIAL TREATMENT FOR MENISCAL INJURIES

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Background: Meniscal injuries are the most common pathology of the knee and are associated with pain, stiffness, and localized swelling. Meniscal damage is a frequent finding on MRI images of knee osteoarthritis (OA).¹ Efforts to repair meniscal damage have been largely unsuccessful and do not prevent the progression of degenerative changes that lead to knee OA.² The Wnt signaling pathway has been shown to be regulated during meniscal development,³ suggesting that manipulation of this pathway may influence the regenerative capacity of the meniscus. Lorecivivint (LOR; SM04690) is an intra-articular (IA), small-molecule CLK2/DYRK1A inhibitor that modulates the Wnt pathway.⁴

Objectives: LOR was evaluated in preclinical studies to determine its protective and anabolic effects in ex vivo explants and in a rat model of chemically induced inflammatory meniscal degeneration.

Methods: Effects of LOR (30 nM) on matrix metalloproteinase (MMP) expression in cultured rat menisci treated with IL-1B were measured by qRT-PCR. In vivo, LOR activity was evaluated in a rat model of monosodium iodoacetate (MIA) injection-induced inflammatory meniscal degeneration. A single IA injection of MIA was immediately followed by a single IA injection of LOR (0.3 ug) or vehicle. Knees were harvested on Days 1, 4, and 11 and menisci were isolated. Anti-inflammatory effects were evaluated by qRT-PCR for *TNFA* and *IL6* expression. Meniscal protection was evaluated by qRT-PCR for MMPs and aggrecanase. Anabolic effects were evaluated by qRT-PCR for collagens.

Results: In ex vivo meniscal explants, LOR inhibited expression of *MMP1*, *MMP3*, and *MMP13* compared with DMSO ($P<0.01$). In vivo, LOR significantly decreased expression of MMPs and aggrecanase ($P<0.05$) and reduced expression of inflammatory cytokines *TNFA* and *IL6* compared with vehicle in the rat model of inflammatory meniscal degeneration at Day 4 after MIA injection. Additionally, LOR increased expression of collagen types I, II, and III at Day 11 after MIA injection (Figure 1).

Conclusion: LOR exhibited protective effects in the meniscus ex vivo and in vivo by reducing catabolic enzyme expression compared with control. Anti-inflammatory effects of LOR were demonstrated by inhibition of inflammatory cytokine expression. Compared with vehicle, LOR increased collagen expression in vivo, indicating potential meniscal anabolic effects. These data support further investigation of LOR as a potential structure-modifying treatment for meniscal injuries.

Figure 1. LOR increased collagen gene expression in vivo. A single IA injection of monosodium iodoacetate (MIA, 3 mg) was immediately followed by a single IA injection of LOR (0.3 µg) or vehicle at 10 weeks of age. Knees were harvested on Days 1, 4, and 11 after injection and menisci were isolated. Gene expression was measured by qRT-PCR. N=3, Mean ± SEM. *P<0.05, one-way ANOVA

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POS0373

LIRAGLUTIDE HAS POTENT ANTI-INFLAMMATORY AND ANTI-CATABOLIC IN VITRO ACTIVITIES IN OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is an age-related joint disease which provokes chronic pain and limits mobility. The disease progression is associated with inflammatory responses and cartilage degradation. Both chondrocytes, the only cell type present in cartilage, and macrophages from the synovium, play a major role in OA pathophysiology. Liraglutide is a Glucagon-Like Peptide-1 Receptor (GLP-1R) agonist widely prescribed for the treatment of type 2 diabetes. Interestingly, anti-inflammatory properties of the GLP-1 pathway have been reported in various diseases outside diabetes.

Objectives: We evaluated the anti-inflammatory and anti-catabolic effects of Liraglutide in two *in vitro* models relevant to OA by evaluating surrogate markers of inflammation, cartilage matrix proteolysis and differentiation.

Methods: Lipopolysaccharide (LPS)-stimulated murine Raw 264.7 macrophages were treated with 10 concentrations (6.6nM-3.4µM) of Liraglutide for 24h. Anti-inflammatory activity was evaluated by the production of nitric oxide (NO) and prostaglandin E₂ (PGE₂) using Griess reaction and ELISA, respectively. Interleukin 1β (IL-1β)-stimulated mouse articular chondrocytes were treated with Liraglutide (6.6nM-3.4µM) for 24h. Production of IL-6, matrix metalloproteinase-3 (MMP-3) and glycosaminoglycans (GAG) was measured by ELISA and GAG assay, respectively. RT-qPCR analyses were performed with three selected concentrations of Liraglutide (13.3nM, 53.1nM and 1.7µM) on both cell types to assess the expression of a panel of genes related to inflammation (IL-6, TNF, iNOS), M1/M2 macrophage phenotype (MCP-1, CD38, ERG-2), catabolism (MMP-13, ADAMTS-5) and differentiation (Sox9, Col2a1, Acan).

Results: Liraglutide induced a dose-dependent inhibition of the LPS-induced production of NO (IC₅₀=45nM) and PGE₂ (IC₅₀=54nM) in macrophages. Moreover, IL-6 and TNF gene expressions were significantly and dose-dependently decreased in Raw 264.7 cells treated with Liraglutide compared to LPS alone. Interestingly, there was a significant dose-dependent reduction of MCP-1 and CD38 (M1 marker) gene expression in cells treated with the 3 doses of Liraglutide compared to LPS alone while we observed a dose-dependent increase of ERG-2 (M2 marker) gene expression induced by Liraglutide. Liraglutide significantly dose-dependently reduced the IL-1β-induced release of IL-6 (IC₅₀=38nM), MMP-3 (IC₅₀=56nM) and GAG (IC₅₀=47nM) in chondrocytes. Additionally, Liraglutide treatment dose-dependently decreased the IL-1β-induced gene expression of iNOS, MMP-13 and ADAMTS-5. Finally, IL-1β decreased gene expression of Sox9, Col2a1 and Acan differentiation markers, which was rescued in a dose-dependent manner by Liraglutide (Table 1).

Conclusion: A shift in M1/M2 macrophage phenotype and the inhibition of chondrocyte expression of several mediators involved in inflammation and cartilage degradation explain, at least in part, our previous results from rodent osteoarthritis models that showed an analgesic, anti-inflammatory and anti-degradative

Table 1. Gene expression results (fold change) in Raw 264.7 murine macrophages or mouse primary articular chondrocytes

Cell type	Markers	Vehicle	Vehicle	Liraglutide (nM)		
				13.3	53.1	1700
Macrophages	- LPS					
	IL6	1.0±0.2*	63.6±7.1	58.8±6.5	36.3±8.4*	30.8±2.6*
	TNFα	1.0±0.2*	26.8±4.9	17.5±2.6*	9.5±1.8*	4.1±2.1*
	MCP1	1.1±0.6*	91.7±11.2	67.8±4.3*	47.3±6.1*	25.1±5.5*
	CD38	1.1±0.6*	103.5±22.3	73.2±12.3*	44.6±8.9*	17.4±3.3*
	ERG2	1.0±0.3*	0.3±0.1	1.1±0.5*	2.1±0.6*	3.1±0.2*
Chondrocytes	- IL1β					
	iNOS	1.0±0.2*	47.8±17.6	34.2±15.5	18.9±8.2*	11.8±2.9*
	MMP13	1.0±0.2*	9.8±2.0	7.6±1.0	4.6±0.7*	2.5±0.4*
	ADAMTS5	1.2±0.8	2.7±0.6	2.0±0.6	1.6±0.3	1.1±0.2
	Sox9	1.0±0.1*	0.4±0.2	0.4±0.2	0.6±0.0	0.7±0.2
	Col2a1	1.0±0.2*	0.3±0.1	0.3±0.1	0.5±0.3	0.8±0.3*
	Acan	1.2±0.8*	0.2±0.1	0.1±0.0	0.5±0.1*	0.7±0.2*

* p<0.05 vs LPS or IL1β alone, n=4

effect of Liraglutide. The fact that Liraglutide is already safely prescribed in another indication allows us to foresee a first trial in humans in the short term.

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POS0374

SENESCENCE DID NOT ALTER THE CHONDROPROTECTIVE EFFECT OF EXTRACELLULAR VESICLES FROM MESENCHYMAL STROMAL CELLS

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Background: Age is the most important risk factor in degenerative osteoarthritis (OA) and is associated with the accumulation of senescent cells that contribute to functional decline of joint. We previously demonstrated that extracellular vesicles (EVs) from mesenchymal stromal cells (MSCs) largely mediate the therapeutic effect of parental cells in OA.

Objectives: Here, we assessed the impact of senescence on the characteristics of EVs from adipose tissue-derived MSCs (ASC-EVs) and their properties in an *in vitro* model of OA

Methods: ASCs were induced to senescence using 25µM etoposide for 24 hours. Senescence was assessed by quantifying proliferation rate, SA-βGal activity, nuclear γH2AX foci number, phalloidin staining and expression of cyclin dependent kinase inhibitors (CDKI) (RT-qPCR). ASC-EVs were isolated by differential ultracentrifugation and characterized by size, concentration, total protein content, structure (cryo-TEM) and immunophenotype. *In vitro* OA model used chondrocytes isolated from OA patients, which were stimulated with IL1β for 48h before culture with ASCs or ASC-EVs for 7 days. Expression of chondrocytic and inflammatory markers was quantified by RT-qPCR and SASP factors were quantified by ELISA in supernatants.

Results: Senescence-induced ASCs experienced growth arrest and increase of SA-βGal staining, of p21 CDKI expression, of nuclear γH2AX foci, of stress fibers and of several SASP factors (IL6, IL8, MMP3) confirming the expression of main senescence features. Senescent ASCs produced 4-fold more EVs than healthy ASCs and senescent ASC-EVs were larger. *In vitro*, both healthy and senescent ASCs decreased fibrotic markers (*type III COLLAGEN*), catabolic and hypertrophic markers (*MMP3*, *MMP13*, *AP*) and increased *COX2* expression in OA chondrocytes. By contrast, healthy ASCs decreased the expression of *IL6* while senescent ASCs highly increased *IL6*. Looking at the role of ASC-EVs on OA chondrocytes, we found out that both healthy and senescent ASC-EVs were able to increase the expression of *AGG* and *type II COLLAGEN* while they decreased the expression of *MMP13*, *AP*, *type X COLLAGEN*, *HMOX1* and *IL6*. Finally, healthy and senescent ASC-EVs decreased the number of SA-βGal positive chondrocytes but did not impact the expression of p21 in IL1β-induced chondrocytes.

Conclusion: Our results indicated a chondroprotective effect of ASC-EVs, independently of the senescent state of parental cells and suggested that EVs might act through different mechanisms than ASCs, which warrants further investigation